Dispatches

Radiation Resistance: Resurrection by Recombination

Adaptation to extreme desiccation has conferred extraordinary radiation resistance on the bacterium *Deinococcus radiodurans*. How this organism is able to reconstruct a genome shattered by γ rays has now been revealed.

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The lethal effects of ionising radiation are well known and widely used for sterilising food and medical supplies. The isolation in 1956 of a bacterium, now known as *Deinococcus radiodurans*, from meat that had been treated with 4000 Gy of γ radiation, was therefore very surprising [1] and has continued to puzzle bacteriologists and molecular biologists ever since. Probably the most important consequence of exposure to ionising radiation is DNA damage, particularly the induction of double strand breaks. To put the radiation resistance of *D. radiodurans* in context (Figure 1), the dose of γ rays required to kill two-thirds of a culture of standard laboratory *Escherichia coli* results in the genome of the cells being broken into fragments over half a million basepairs in length; at the dose required for the same degree of killing of *D. radiodurans*, the genome is broken into pieces on average only ten thousand base pairs long [2]. Despite this catastrophic destruction, *D. radiodurans* is able to reconstitute intact and apparently unmutated chromosomes within only six hours, but the nature of the repair mechanism used to perform this extraordinary feat has been unclear. Now, with a series of elegantly simple experiments, Miroslav Radman's group [3] have discounted all of the formal possibilities predicted by current theory and shown that genome reconstitution in *D. radiodurans* takes place as a two-stage process, the first of which involves a novel mechanism (Figure 2).

A key observation is that the repair process is accompanied by extensive DNA synthesis. Unusually, most of this newly synthesised DNA is double stranded, in contrast to that generated by normal semi-conservative DNA replication in which only one of the two strands is new. Further, it appears that these blocks of newly synthesised DNA, which are used to join the fragments of the genome together, are initially formed as single strands that are then brought together. This suggested a mechanism that Radman terms extended synthesis-dependent strand annealing.

Classical synthesis-dependent strand annealing is a well-established model of homologous recombination between two sequences which is able to explain instances of recombination where crossovers are not seen [4]. It involves the extension of the 3′ end of the DNA on one side of a double-strand break by DNA synthesis on a homologous template or donor. The newly synthesised strand is then unwound from the donor and is now able to return to its original position to bridge the double-strand break. Extended synthesis-dependent strand annealing is a related model but differs in that both ends of the broken DNA fragments acquire long 3′ tails by DNA synthesis using a homologous chromosomal fragment as a template (Figure 2B). Once released from their donor, presumably by an as yet unidentified helicase, these newly synthesised 3′ tails act like glue to piece the genome back together (Figure 2C).

The length of the newly synthesised DNA is notable and is likely to facilitate accurate assembly of the chromosome. At 20–30 kilobases, its formation is quite likely to require multiple cycles of invasion and extension using more than one donor. This extended synthesis-dependent strand annealing step is preparatory to the second phase of repair (Figure 2D), in keeping with an earlier suggestion of Daly and Minton [5], in which final chromosomal assembly results from resolution of regions of overlap by classical RecA-dependent homologous recombination. To work, extended synthesis-dependent strand annealing therefore requires two or more copies of the genome — *D. radiodurans* contains four to ten copies [6] of

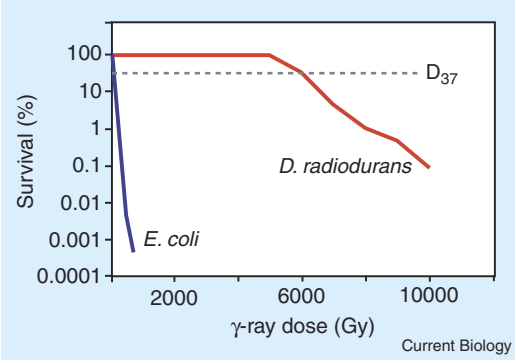

Figure 1. Survival of *D. radiodurans* and *E. coli* following exposure to γ radiation.

The *D. radiodurans* curve is shown in red and the *E. coli* curve in blue. The D_{37} level is that at which two thirds of a population are killed and is indicated by the grey dotted line. (Adapted from [15].)

its 3.3 megabase genome — and for the DNA breaks to be generated randomly.

Many details of the mechanism of extended synthesis-dependent strand annealing are still to be elucidated, as are the interactions of the repair and recombination machinery with the highly condensed nucleoid architecture of the *D. radiodurans* genome [7] and with the mechanisms that protect the DNA ends from excessive nucleolytic degradation [8], both of which are important for radiation resistance. Furthermore, DNA double-strand breaks are only a part of the repertoire of damage caused by ionising radiation. Much oxidative damage to bases also arises in consequence of the generation of highly reactive free radicals from the ionisation of water by γ rays. Such base damage frequently presents a block to DNA synthesis and might therefore be expected to significantly hamper the highly DNA synthesis-dependent repair reaction described by Radman's group [3].

While some bacteria are able to respond to damage by employing specialised translesion polymerases, for example polymerase IV and polymerase V in *E. coli*, such a potentially mutagenic mode of DNA synthesis would seem imprudent in *D. radiodurans*. Indeed, the *D. radiodurans* genome appears to encode only three polymerases [9], one of which is involved in replication while the other two are involved in homology-directed repair [3,9,10]: no homologues of the *E. coli* translesion polymerases, or an SOS regulon, are present [11]. *D. radiodurans* does, however, possess a number of adaptations that reduce the toxic effect of free radicals. These possibly include the carotenoid pigments, which give the bacterial colonies their characteristic red colour, and an enormous array of DNA glycosylases and Nudix hydrolases, enzymes that are able to remove oxidatively damaged bases from DNA and from the nucleotide pool respectively [9]. Thus, prevention and repair of base damage are likely to be important contributors to the radiation resistance of *D. radiodurans* alongside its facility in stitching its genome back together.

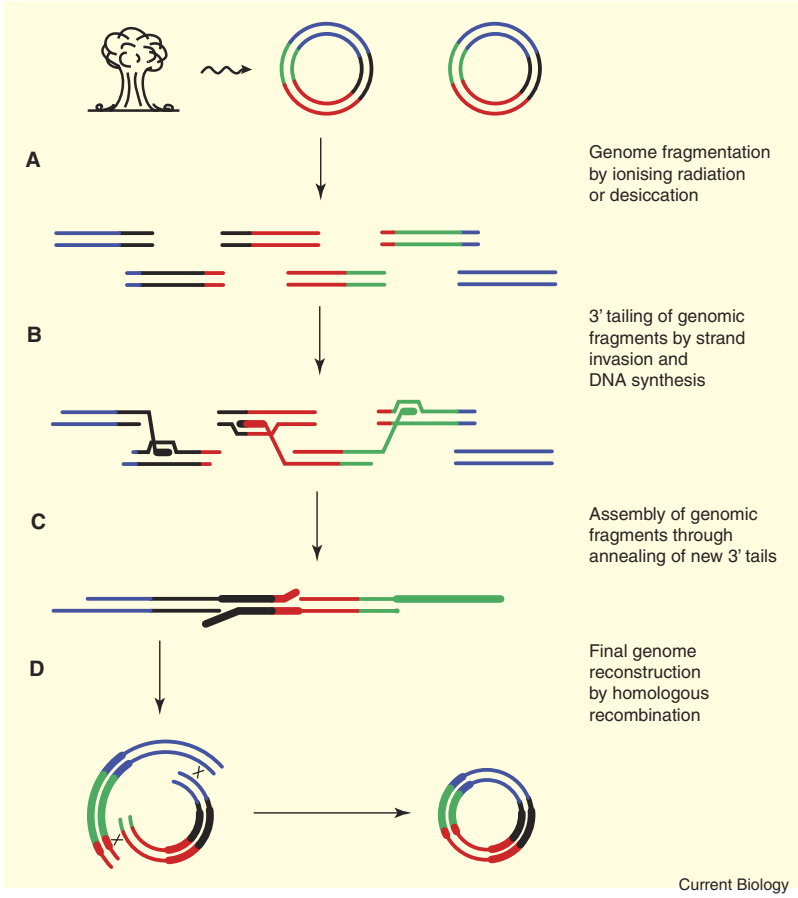

Figure 2. A model for the repair of a shattered genome in *Deinococcus radiodurans*.
(A) Multiple DNA double-strand breaks are introduced randomly into multiple copies of the genome by ionising radiation or desiccation. Two genome copies, each with four contiguous regions (blue, black, red and green), are shown for clarity. (B) 3′ overhangs are generated by limited 5′ degradation. These can invade other overlapping genomic fragments. DNA synthesis, by PolA, results in the formation of long 3′ tails. The newly synthesised DNA is shown as a thicker line. (C) These tails allow annealing of adjacent genomic fragments, the long region of overlap promoting accuracy of assembly. (D) An intact chromosome, comprising a patchwork of old and newly synthesised DNA, is then formed by RecA-mediated homologous recombination. (Adapted from [3].)

Why did such remarkable radiation resistance evolve? After all, the most naturally radioactive places on Earth result in exposures of less than 400 mGy per year. The clue comes from the habitats in which *Deinococci* can be found, which are often extremely dry [12], and it seems likely that it is this insult to which the organism has adapted. Desiccation results in extensive DNA damage and breakage [13] that renders the bacterium, as Radman puts it, "clinically dead". Addition of water and ions, however, results in its 'resurrection' and the remarkable reassembly of its genome.

Interestingly, bacteria are not alone in exhibiting extreme radiation resistance. There are species of archaea that show a similar hardiness [2]. More surprising are the eukaryotic cells that are also able to withstand several thousand Gy of γ radiation, including the well-studied slime mould *Dictyostelium discoideum* [14], which raise the intriguing possibility that some eukaryotes have also been able to adapt well-established repair mechanisms to deal with extreme DNA damage.

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Human Populations: Houses for Spouses

Patterns of genetic variability in human populations are profoundly influenced by social organisation, including lifestyle, language, religion and social status. A nice illustration is seen among societies that have specific rules about who can marry whom.

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Anyone who studies population genetics learns one thing quickly: the field is full of unrealistic assumptions. The real genetics of real populations is so complex that we need to start simplistically: in basic models to explain the distribution of genetic diversity, we assume, for example, that populations are infinitely large, that every individual has an equal chance of mating with every other, that there is no migration, and that natural selection is not acting. Watch any nature programme on television, however, and it soon becomes clear that none of these assumptions holds true. For any animal species in the real world, we need to consider population size, geographical distance and landscape, resources, individual fitness and social organisation.

Social organisation among animals can be surprisingly complex, but in humans it is particularly so because of uniquely human aspects of culture: language, religion and political status are interconnected, and characterise each society. These factors have a major impact on patterns of genetic diversity because they profoundly affect choice of marriage partner. All societies have implicit or explicit rules governing marriage, and also where the new family will settle and raise their children.

The effects of traditions concerning location of the marital home have been investigated by geneticists, exploiting segments of the genome that are inherited through only one sex — namely, mitochondrial DNA (mtDNA) and the Y chromosome. About 70% of modern human societies [1,2] practice patrilocality, where the woman moves to the man's place of residence after marriage. At the local scale, at least [3], this small-scale migration of females reduces the geographical differentiation of maternally inherited mtDNA, while the relative immobility of males has the opposite effect on the paternally inherited Y chromosome [4]. In some populations it is the men who move, and the women who stay put, and in these matrilocal groups the opposite patterns of mtDNA and Y diversity are seen [5].

The same uniparentally inherited markers have now been used, as Chaix *et al.* [6] report in this issue, to illuminate the effects of marriage rules, and how these differ between societies with different lifestyles. Central Asia is home to both pastoral nomads and sedentary farmers (Figure 1). While farming populations are organised into extended or nuclear families, pastoral populations are made up of a hierarchy of descent groups, in which individuals belong to lineages (in which they can define with certainty their links through a common ancestor), clans (groups of lineages where common ancestry is claimed, but not certain) and tribes (groups of clans which share language, culture and territory). The rules